Environmental Health Perspectives
Vol. 34, pp. 47-57, 1980

Experimental Approaches for Exposure to Sized Glass Fibers

by David M. Bernstein,*† Robert T. Drew,* and Marvin Kuschner‡

A number of studies have shown that glass fibers induce both malignant mesothelioma and fibrosis in rats and that these reactions may be primarily a function of the physical properties of the fiber. However, these studies were carried out with fibers having broad size distributions and used methods of administration which bear little resemblance to the way man is exposed.

To better characterize the health effects of glass fibers, techniques have been developed to expose rats to glass fibers of defined sizes by intratracheal instillation of aqueous suspensions and by "nose only" inhalation exposure, and to determine the deposition, translocation, and ultimate fate of these fibers in the rat. The fibers have known size distributions with geometric mean diameters of 1.5 μm (σ_g = 1.1) and lengths of either 5 μm (σ_g = 1.49) or 60 μm (σ_g = 3.76). The fibers have been activated with neutron irradiation. Of the several resulting radionuclides, ^{65}Zn appeared to be the most suitable for long-term clearance studies by use of *in vivo* whole body radioassay techniques.

A fluidized bed aerosol generator has been developed to expose rats by "nose only" inhalation to approximately 500 fibers/cm^3. The generator and exposure system permits reuse of fibers which pass through the exposure chamber and produces no significant alteration of the fiber size distribution.

Rats were exposed by intratracheal instillations to 20 mg of the longer fibers and to equal numbers (2 mg) and equal mass (20 mg) of the shorter fibers. Through approximately 19 weeks little difference was observed in the whole rat clearance rate of long versus short fibers in the initial exposure group. Histopathology, however, showed differences at this time with the short fibers apparently successfully phagocytized by alveolar macrophages and cleared to the lymph nodes, while the long fibers were not.

Introduction

Glass fibers have been shown to induce malignant mesothelioma when introduced directly into the pleural cavity of rats (*1*). Suspensions of glass fibers have also produced pulmonary fibrosis when instilled into the trachea (*2*). These studies indicated that such tissue-damaging reactions to fibers may be primarily a function of their geometry, with long (>20 μm), thin (<2 μm) fibers thought to be the most reactive and shorter (<5 μm) fibers less active or inactive. However, the studies above, along with most other studies on biological effects of fibers, have used fibers with broad particle size distributions with respect to both length and diameter. In addition, the modes of administration bear little resemblance to the way man is exposed, leaving some doubt as to which properties of the fibers caused the fibrosis and mesothelioma.

To better characterize the health effects of fiber glass, a study has been implemented to determine the deposition, translocation, and ultimate fate of fibers of defined size distributions, when introduced into the respiratory tract of rats. The glass fibers have known size distributions with a geometric mean diameter of 1.5 μm and a length of either 5 or 60 μm. The rats have been exposed to these fibers by intratracheal instillation of aqueous suspensions and by "nose only" inhalation exposure. While it is recognized that intratracheal instillation bears little resemblance to the way man is exposed, the techniques of inhalation exposure of glass fibers in this study are considerably more complex. Therefore, intratracheal instillation was used as an initial step in

*Medical Department, Brookhaven National Laboratory, Upton, New York 11973.
‡School of Medicine, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794.
†Author to whom reprint requests should be addressed.

Table 1. Manufacturers' fiber size characterization.

	1.5 × 5 μm		1.5 × 60 μm	
	Diameter, μm	Length, μm	Diameter, μm	Length, μm
Average	2.08	6.0	1.94	50.8
Geometric mean	2.06	5.7	1.91	43.9
Median	2.08	5.7	1.87	56.0
Standard deviation	0.29	1.8	0.35	24.7
Minimum	0.73	2.0	0.93	5.7
Maximum	3.02	15.2	3.63	224.5
N	600		600	

studying the health effects of known quantities of glass fibers deposited in the lungs of rats. In addition, exposing the animals by both methods affords an opportunity to evaluate the relative merits of intratracheal instillation as compared to nose exposure.

Table 2. Size characterization from fiber study.

N	Geometric mean	Geometric SD
250	5.1	1.49
500	54	3.76

This report describes the methods and techniques developed for both routes of exposure, and presents the clearance data and histological results from the intratracheal instillation exposure.

Materials and Methods

Glass Fibers

The fibers, manufactured by Johns Manville, Inc., were either 1.5 × 5 μm or 1.5 × 60 μm in size. The

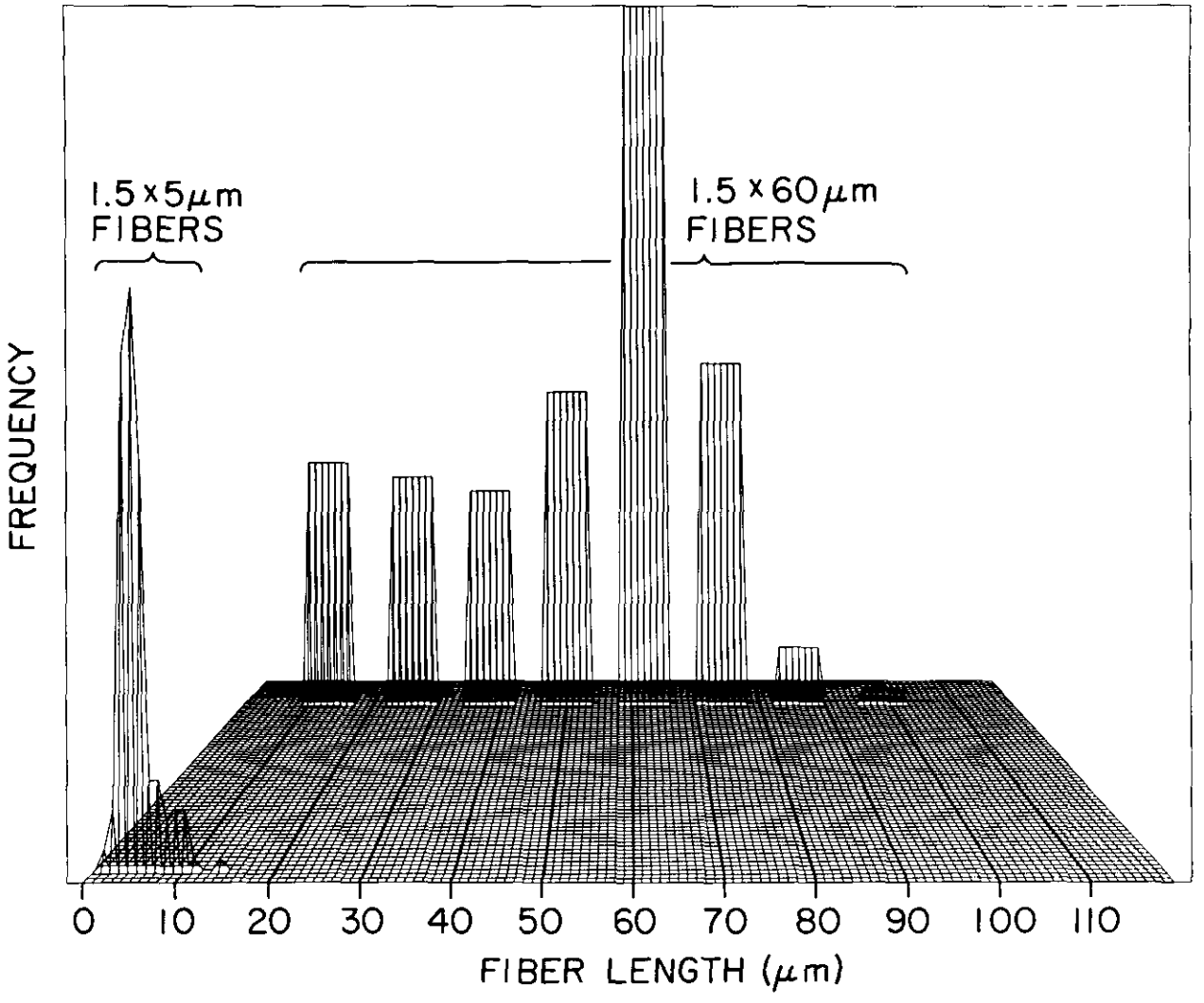

FIGURE 1. Length distributions of the 1.5 × 5 μm and 1.5 × 60 μm glass fibers.

procedure involved first machining a single die to produce a fiber of 1.5 μm diameter. After a continuous strand was threaded through a series of channels in an acrylic block, the channels were embedded with methacrylate, and the block cut on a microlathe to various lengths. The acrylic was then dissolved away with methyl ethyl ketone. Residual contaminants were removed by low temperature ashing, followed by further washing of the fibers.

The size distributions of the fibers were determined by the manufacturer by measuring the length and diameter of 600 fibers in each size group. The length size distributions were confirmed in our laboratory to ensure that no breakage of fibers occurred in transportation or handling. The results of these measurements are summarized in Tables 1 and 2. While the length size distributions are not monodisperse, Figure 1 shows that they do not overlap significantly.

Animals

These studies were conducted with male Fisher 344 rats purchased from Microbiological Associates. The animals were approximately 10 weeks old at the time of exposure. They were housed in wire cages, allowed food and water ad libitum, and maintained on a 12-hr light cycle at 22 ± 2°C, 50% RH.

Methods

To quantitate the deposition and clearance of the fibers in the rats the fibers were neutron-activated for 18 hr at a flux of ~ 1×10^{14} neutrons/cm^2/sec. After the activated fibers decayed for approximately 12 weeks, the longer-lived radionuclides ^{65}Zn, ^{60}Co, ^{152}Eu, ^{46}Sc, and ^{124}Sb remained. Of these ^{65}Zn was determined to be the best tracer for the fibers. Leaching studies in saline at 37°C for 24 hr indicated that cobalt is readily removed from the fibers.

The isotopes present in the fibers were assayed by γ-ray spectroscopy. The detection system consists of two 6 × 3 in NaI (Tl) scintillation crystals multiplexed into a Tracor Northern 1705 multichannel analyzer. The spectra are stored on magnetic tape and the relative amounts of each radionuclide in the fibers determined with the aid of the computer program PARANA (*3*, *4*) (Pulse Height Analyses for Radionuclide Assay) which performs least squares regression of the multicomponent γ-ray spectra. The major components of the glass fibers as determined by the manufacturer and the isotopes detected by instrumental neutron activation analysis are listed in Tables 3 and 4, respectively.

Intratracheal Instillation Exposure. A procedure for the pulmonary instillation of particle suspensions was developed for use in this laboratory. The procedure involves anesthesia of the animal, followed by depression of the tongue and illumination of the deep pharynx with a fiberoptic laryngoscope. A bevelled Teflon tube is inserted into the trachea to a level just cephalic to the first bronchial bifurcation. Injection of the material to be delivered is rapid via a No. 19 gauge hypodermic needle inserted directly into the tracheal tube.

Four groups of rats were instilled with fibers as outlined in Table 5.

Rats in group 2 thus were given approximately the same number of fibers as rats in group 1 and rats in

Table 3. Elemental composition of glass fibers (Johns-Manville Batch JM-753 Glass).

Major Components	Content, %
SiO_2	63.2
B_2O_3	5.4
Al_2O_3	5.5
Fe_2O_3	0.1
Na_2O	14.8
K_2O	1.1
CaO	6.0
MgO	3.1
F_2	0.7
SO_3	0.2

Table 4. Isotopes detected by instrumental neutron activation analysis 10 days after irradiation using Ge(Li) detection system.

Isotope	Isotope half life
Sodium-24	15.0 hr
Bromine-82	35.3 hr
Lanthanum-140	40.2 hr
Gold-198	2.7 d
Calcium-47	4.5 d
Lutetium-177	6.7 d
Chromium-51	27.7 d
Cerium-141	32.5 d
Hafnium-181	42.4 d
Iron-59	44.6 d
Antimony-124	60.2 d
Strontium-85	65.2 d
Scandium-46	83.8 d
Zinc-65	243.7 d
Cesium-134	2.06 yr
Cobalt-60	5.3 yr
Europium-152	13.0 yr

Table 5. Instillation of fibers.

	Fiber size, μm	Amount, mg	Saline, cm^3
Group 1	1.5 × 60	20	0.5
Group 2	1.5 × 5	2	0.5
Group 3	1.5 × 5	20	0.5
Group 4	—	—	0.5

group 3 were given the same weight of fiber glass as those in group 1.

Initially 4 rats/group were instilled with activated fibers and sacrificed 18 to 20 weeks after exposure. An additional 30 rats/group were then instilled with similar doses of fibers with serial sacrifices of 4 rats/group beginning at 6 months and extending through two years. Finally, to ensure that any long-term effects are a result of the fibers and not the small amount of radioactivity present in them, an additional 50 rats/group were instilled with nonactivated fibers.

Inhalation Nose Exposure System. Because of the elaborate process involved in manufacturing the fibers to size, only a few grams are available for all the exposures in this study. Hence special techniques had to be developed to generate high concentrations of fibers in the exposure chamber without breaking the fibers and to conserve the amount of fibers that were available.

A miniature fluidized bed aerosol generator and a specially designed exposure chamber shown in Figure 2 were developed. The fibers were ultrasonicated in ethyl alcohol and deposited on a Teflon filter at the bottom of a glass tube (d = ½ in.). The tube was vibrated, as an airstream was passed up through the filter, creating a fluidized bed of fibers from which individual fibers became airborne. The fiber/airstream was directed through a ^{85}Kr charge neutralizer and into the exposure chamber. The highly polar nature of the glass fibers necessitated a pretreatment with successively nonpolar solvents beginning with ethyl alcohol, methyl ethyl ketone, and finally petroleum ether. This treatment is performed on the fibers in the fluidized bed generator directly before exposure.

The rats were exposed four at a time for 1 hr at a concentration of ca. 500 fibers/cm^3 of air. To conserve fibers, the generator was operated in cycles of 1 min on and 40 sec off, with the settling time of the airborne fibers being sufficiently long that little loss occurred by deposition during the off time. The fibers passing through the chamber were collected on a Teflon filter. These fibers were washed as de-

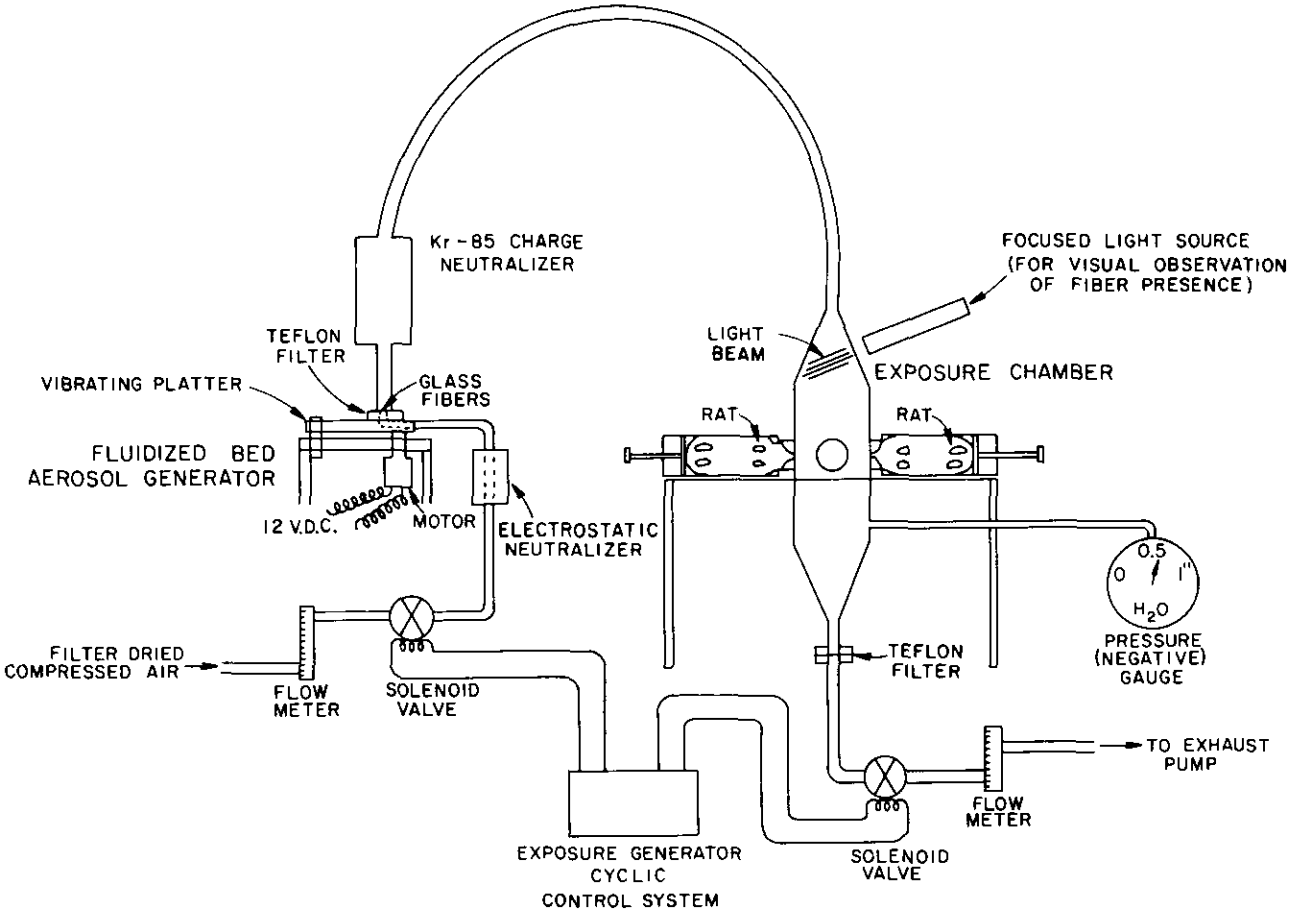

FIGURE 2. Schematic representation of fiber generation and exposure system.

scribed above and kept for reuse. The size distribution of these fibers has been found to be not significantly different from that of the original fibers (Mann-Whitney Statistic, $p < 0.05$).

Necropsy. The necropsy procedure for the rats involved anesthesia followed by exsanguination via the descending abdominal aorta. The lungs were then perfused *in situ* with heparinized isotonic saline through the pulmonary artery, removed, and inflated via an endotracheal tube with glutaraldehyde vapor (20 cm water); and then infused with glutaraldehyde fixation solution through the pulmonary vasculature (20 cm water).

After fixation, the lymph nodes, thymus, and adipose tissue were resected. In addition, the diaphragm, gastrointestinal tract, liver, spleen, kidney, brain, and femur were removed for histological examination and autoradiography to determine the presence of fibers in these tissues.

Tissues were embedded in paraffin, sectioned (6 μm), mounted on a glass slide, and stained with either hemotoxylin and eosin or Trichrome Blue. The refractive index of the fibers is such that the fibers are invisible in the standard Permount mounting media. When mounted in glycerin, however, the fibers are visible by light microscopy.

Autoradiography. An autoradiographic (ARG) technique was adopted for use in localizing the fibers in the tissues. While it is usually easy to locate large quantities of fibers in the lung, the ARG procedure greatly enhances detection of smaller numbers of fibers in other tissues. The beta particles emitted from the radionuclides in the activated fibers are sufficient to expose in one week's time a Kodak NTB-3 emulsion which has been coated on the histological sections. Single activated fibers were clearly identifiable under the exposed emulsion, as seen in the lung section in Figure 3.

Results

Clearance of Glass Fibers

As noted above, cobalt was the only radionuclide found in the supernatant fraction after the fibers were subjected to a saline leaching procedure. From

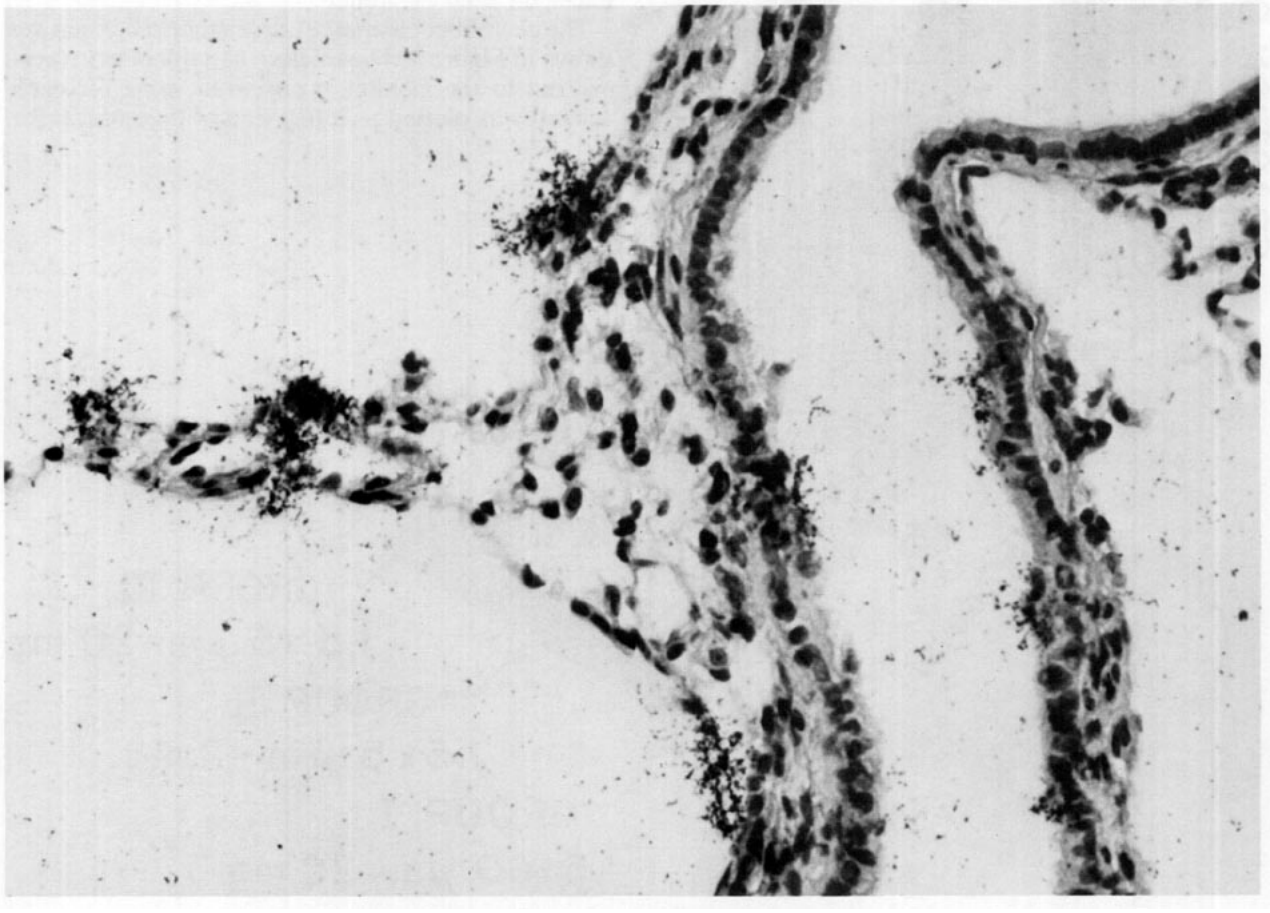

FIGURE 3. Autoradiography of glass fibers (3 × 10 μm) in histological section of rat lung.

the nuclide clearance data and the leaching data, at least two different subsets of tracer nuclides were postulated to be present in the fibers: those securely bound to or in the fibers that would serve as good tracers for the fibers in the rat and those readily removed from the fibers.

The statistical method of factor analysis (5) was applied to the clearance data for the five radionuclides to test this hypothesis. Two independent factors were derived from the data, as can be visualized in Figure 4, with the horizontal axis representing factor 1 (fiber tracers) and the vertical axis representing factor 2 (the surface leachable contaminants). Cobalt stood alone as a leachable contaminant, and the remainder of the radionuclides (Zn, Eu, Sc, and Sb) were all of nearly equal value as tracers for the fibers themselves. Of the elements that were good tracers for the fibers, zinc exhibited the highest activity and was therefore used as a representative tracer for fiber clearance.

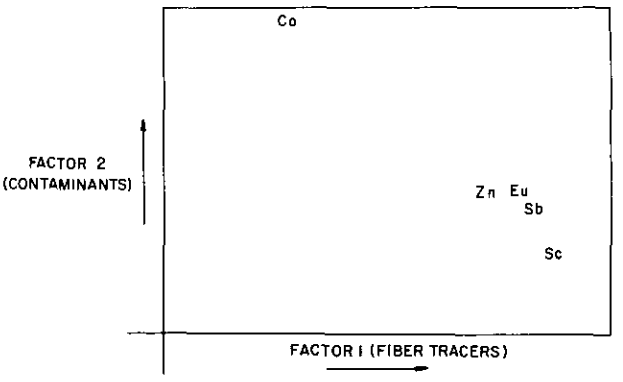

FIGURE 4. Graphical representation of factor analysis results (factor loading).

The glass fiber clearance curves for each group are shown in Figure 5. The relative activity of zinc (normalized to the maximum observed value for each animal) was plotted as a function of time (days) for

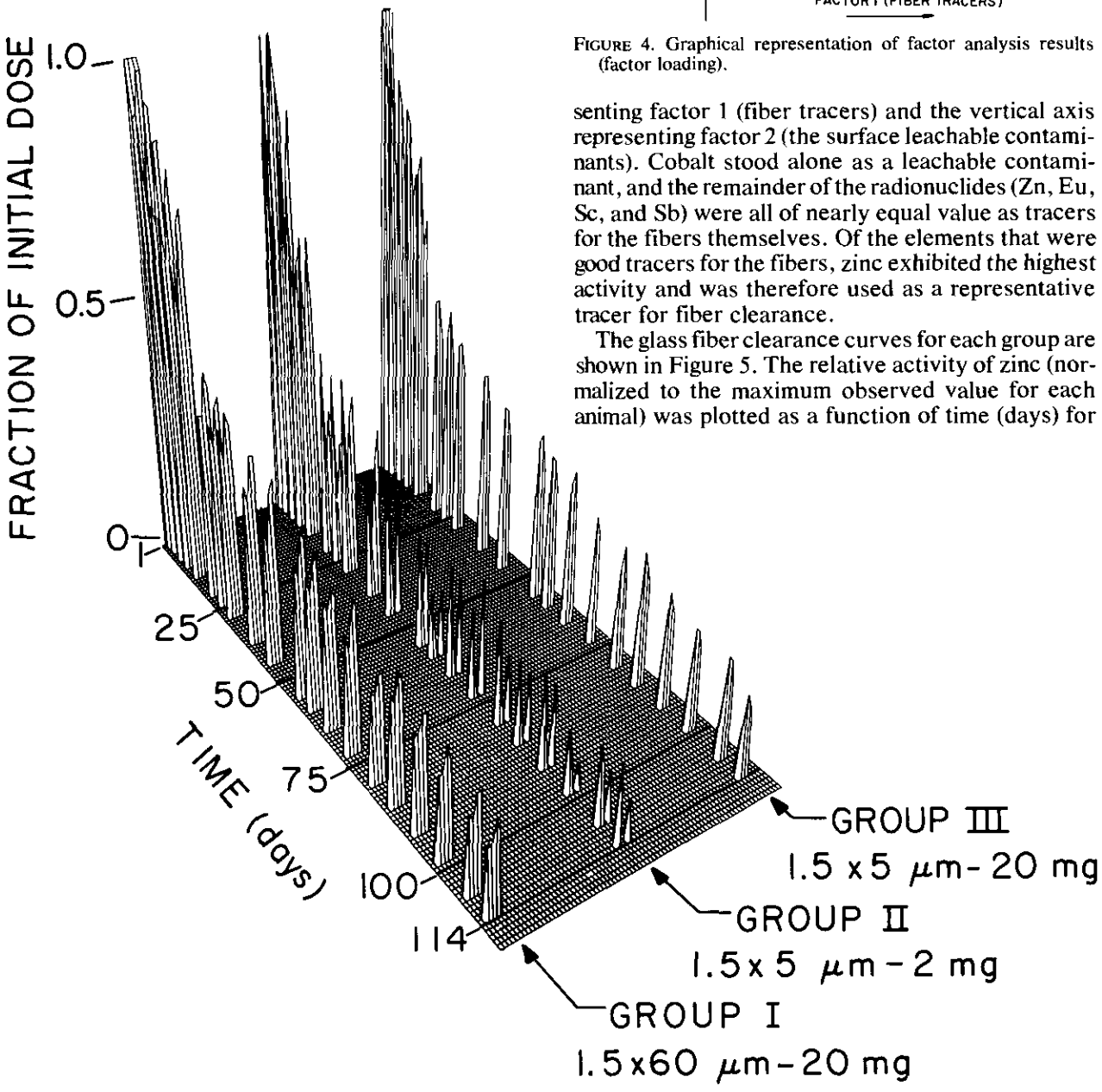

FIGURE 5. Clearance of glass fibers following intratracheal instillation.

each exposure group with the day of exposure designated as day one. The clearance halftimes for each group as estimated by a one component exponential model and the percent of the initial dose which remained at sacrifice (~ 114 days) are given in Table 6. An analysis of variance for repeated measures was performed to test whether the clearance curves differed significantly from one another. The results indicated that groups 1 (1.5 × 60 μm, 20 mg) and 3 (1.5 × 5 μm, 20 mg) were not significantly different from one another, but that group 2 (1.5 × 5 μm, 2 mg) showed a small but significant difference ($p < 0.05$) from groups 1 and 3.

Table 6. Estimates of fiber glass clearance (zinc used as a tracer).

Exposure group	Half times, one component, days	% remaining at ca. 114 days (mean ± S.E.)
Group I 20 mg, 1.5 × 60 μm	35.0	16.3 ± 1.2
Group II 2 mg 1.5 × 5μm	22.5	14.3 ± 7.8
Group III 20 mg, 1.5 × 5 μm	38.5	14.8 ± 1.6

Fiber Translocation and Histological Results

The histological results reported here were from one point in time (17-19 weeks). There were four rats per group. It is important to note that no definite statement can be made about the progression of any of the pathological changes observed, only that they existed at that point in time. The histological sectioning of thin sections (6 μm) often results in the illusion that the fibers are in one plane or that they are shorter than would be expected. Microscopic examination of thick sections reveals that the fibers are, in general, randomly oriented.

The long fibers (1.5 × 60 μm, 20 mg) caused the development of numerous, sharply demarcated, relatively large, foreign body granulomata, with numerous giant cells and fibers (Figs. 6 and 7). Most

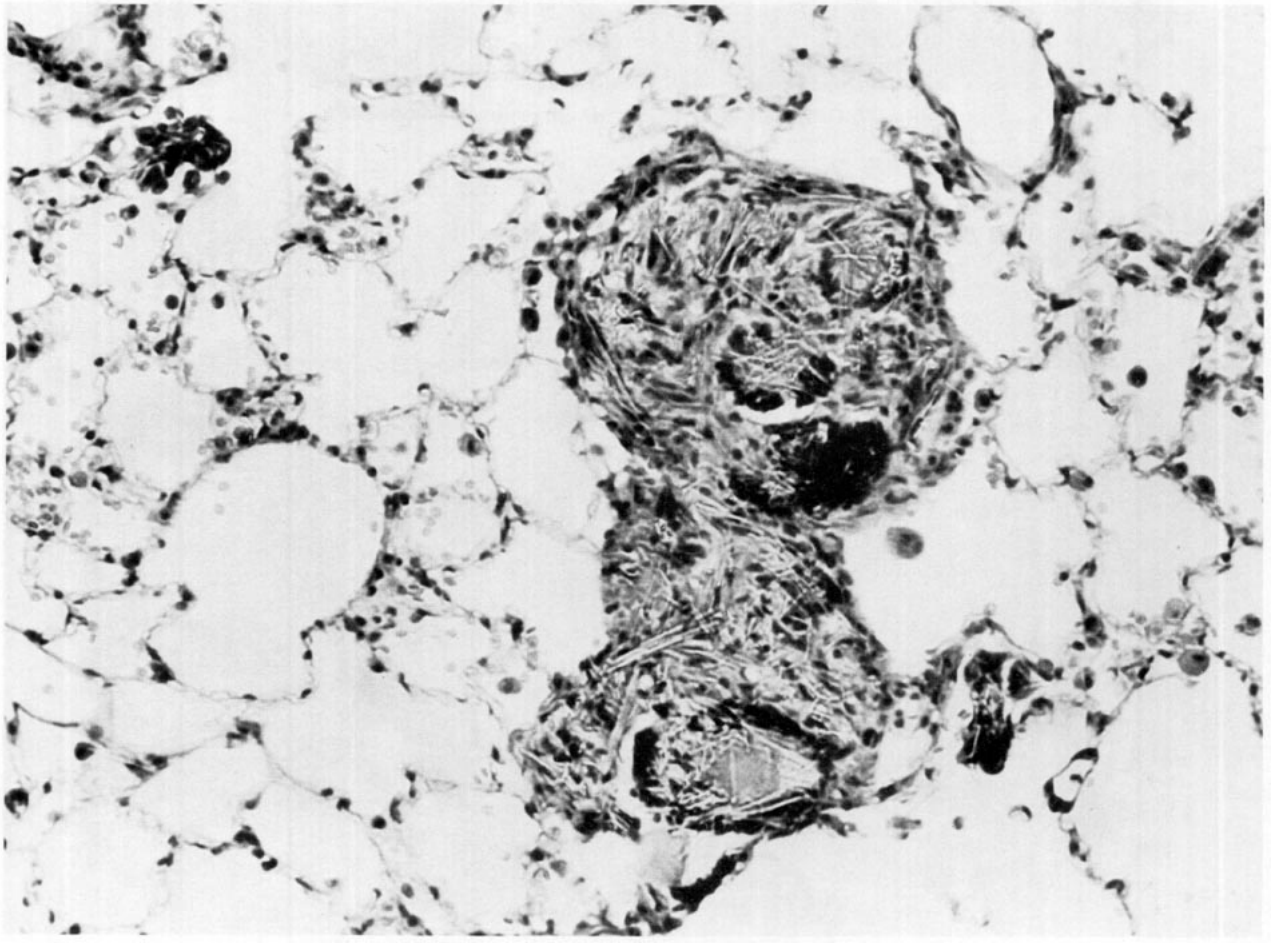

FIGURE 6. Foreign body granulomata in lung; 20 mg, 1.5 × 60 μm exposure group, 139 ×.

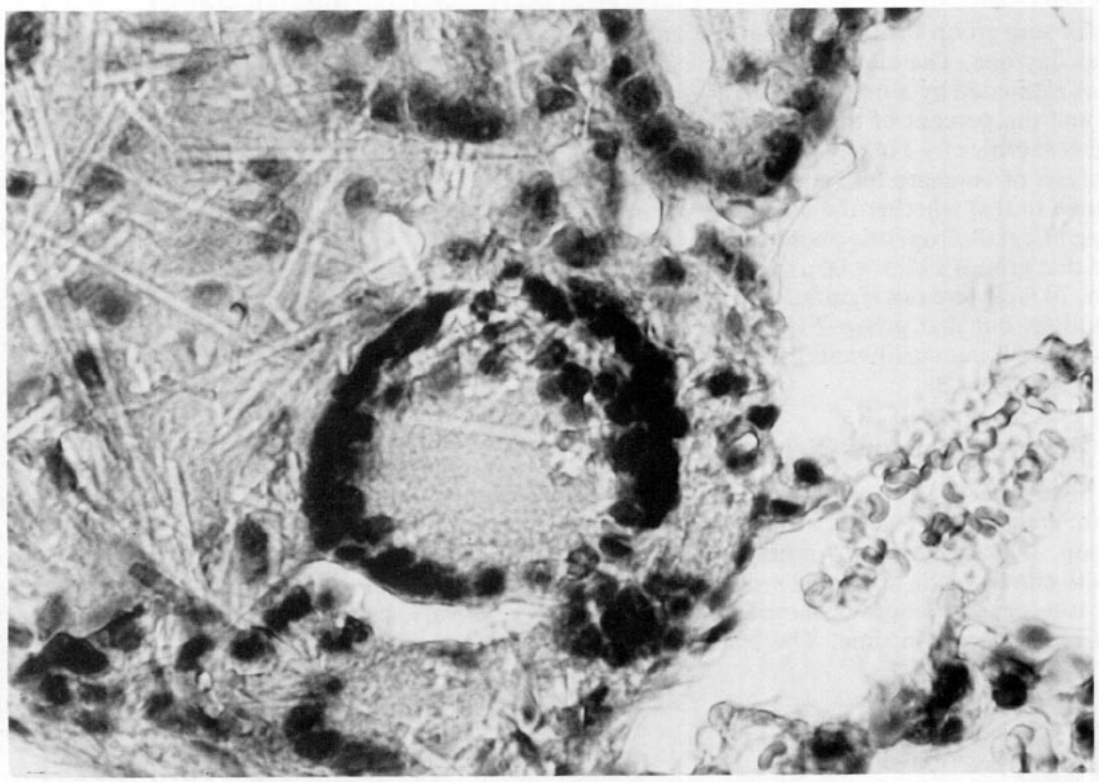

FIGURE 7. Giant cell; 20 mg, 1.5 × 60 μm exposure group, 239 ×.

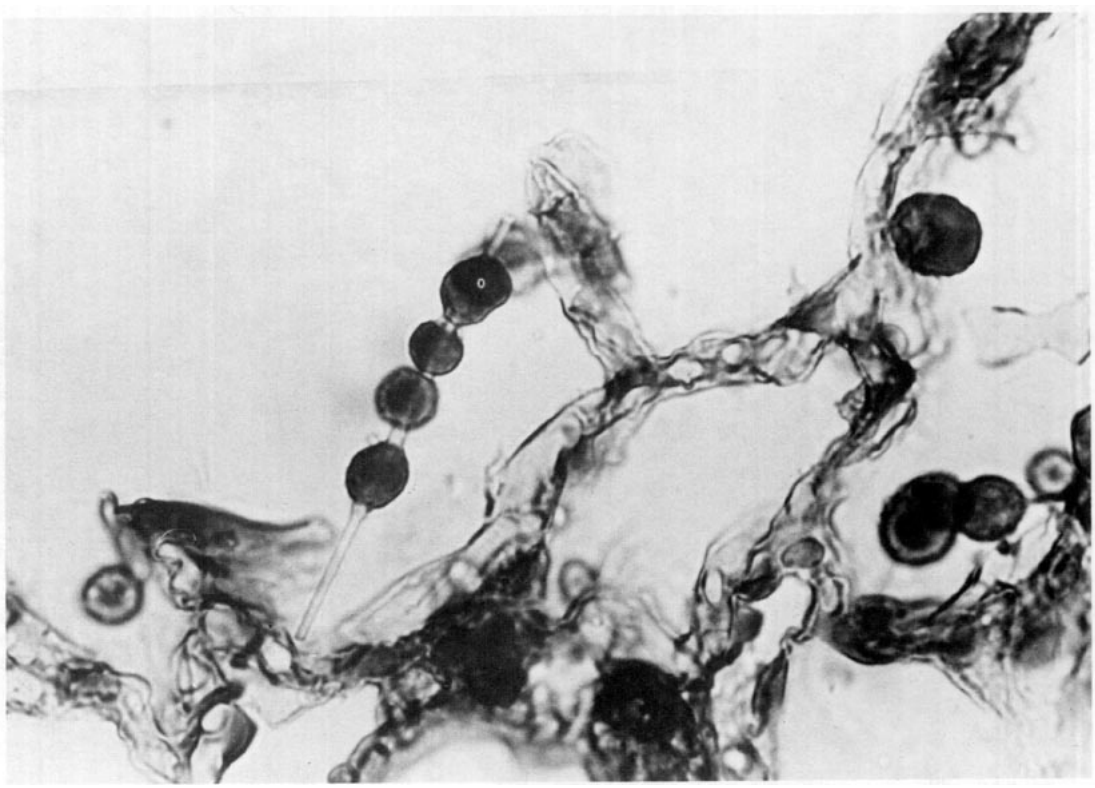

FIGURE 8: 1.5 × 60 μm fiber partially engulfed by macrophages, 239 ×.

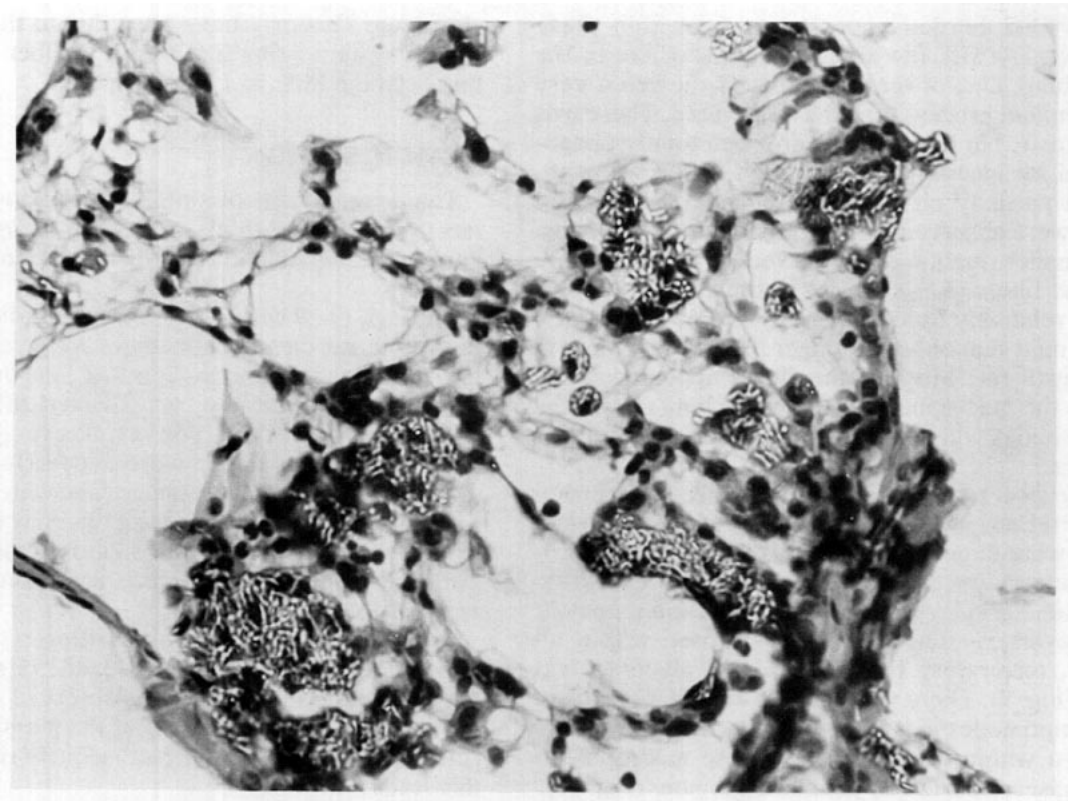

FIGURE 9. Fiber-laden macrophages; 20 mg, 1.5 × 5 μm exposure group, 116 ×.

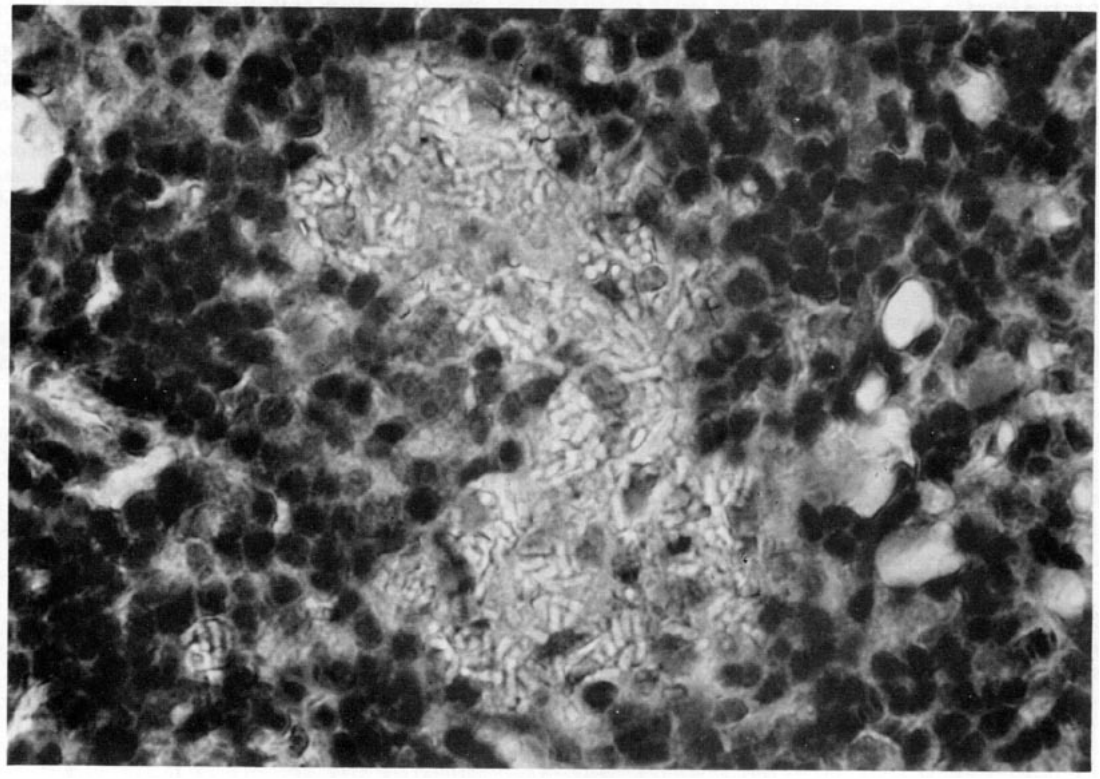

FIGURE 10. Tightly packed aggregates of 1.5 × 5 μm fibers in lymph nodes; 20 mg exposure group, 239 ×.

of the fiber burden appeared to lie within these granulomas, with few fibers seen elsewhere in the distal lung. One of the four animals showed a very much milder process than the other three. There was no fibrosis. No long fibers were seen totally phagocytized by macrophages although a few were observed partially phagocytized (Fig. 8). No 60 μm fibers were observed in the lymph nodes, although a few smaller fibers were observed.

Short fibers (1.5 × 5 μm) at the 2 mg dose produced relatively little alteration in three of the rats, and a mild mononuclear (macrophage) response in the fourth rat. Most of the fibers appeared to lie within the macrophages both in the lung and in the lymph nodes where they occurred in tightly packed clusters.

Short fibers at the 20 mg dose produced a spectrum of alterations with differences between the rats. There were large numbers of fiber-laden interalveolar macrophages. There were small linear and irregular nodular aggregates of fiber-filled mononuclear phagocytes layered along and enclosed within alveolar, respiratory bronchiolar, and alveolar duct walls (Fig. 9). There was no fibrosis. Fibers reached the lymph nodes in large numbers where they could be seen within macrophages. These macrophages were observed in tightly packed aggregates (Fig. 10).

Discussion

At 17-19 weeks after exposure, the major response to the short fibers was to produce macrophage aggregations in the alveoli and lymph nodes. These macrophages contained numerous glass fibers. Except for a few, small, relatively well defined granulomas in the 20 mg group, the 2 mg group produced similar but proportionately less response than the 20 mg group.

The response to the long fibers was notably different. No long fibers were observed in the lymph nodes. It appears that the long fibers did not break extensively. Only a very few pieces of shorter fiber were seen in the lymph nodes; if extensive breakage of the fibers had occurred it is postulated that many more fibers should have been observed.

Few, if any, fibers were found by light microscopy outside the respiratory tissues. The whole rat clearance data would therefore be a good measure of total respiratory (lung and lymph node) clearance. Even though there was an apparent difference in macrophage response to the long versus short fibers, no difference was observed in the total respiratory clearance. This may have been due to the fiber detection system being insensitive to fiber translocation between lung and lymph nodes.

Conclusions

The development of suitable techniques enabled rats to be exposed to glass fibers of defined size by intratracheal instillation and by inhalation (nose) exposure.

Through 17-19 weeks, there was little difference in the whole rat clearance rates of long versus short fibers after exposure by intratracheal instillation. The histopathology, however, showed differences at this time in the rat's response to the long and short fibers. The short fibers were successfully phagocytized by alveolar macrophages and were cleared to the lymph nodes, while the long fibers were not. The long fibers produced a granulomatous response with giant cells that was a characteristic response to many types of foreign bodies.

These studies are continuing with serial sacrifices to be performed at intervals over the rats' lifespan. The progressions of the lesions observed at this time and the further translocation of the glass fibers will better be evaluated with the subsequent results from this study.

The authors wish to thank Mr. Robert Peck and Ms. Patricia Carr for much of the analytical and necropsy work, Mr. James Lehmann for assistance with the intratracheal instillation, and Ms. Gail Schuman for the data management and analysis.

We would also like to thank Dr. Philip Kane for evaluating the histological slides; Dr. Daniel Costa for his efforts in developing the instillation, dissection, and necropsy protocols; and Mr. George Schidlovsky for the autoradiography procedures.

This work is supported by the Thermal Insulation Manufacturers Association and the United States Department of Energy under Contract No. EY-C-02-0016.

REFERENCES

1. Stanton, M. F., and Wrench, C. Mechanisms of mesothelioma induction with asbestors and fiber glass. J. Nat. Cancer Inst. 48: 797 (1972).
2. Wright, G. W., and Kuschner, M. The influence of varying lengths of glass and asbestos fibers on tissue response in guinea pigs. In: Inhaled Particles, IV, W. H. Walton, Ed., Pergamon Press, London, 1977.
3. Pasternack, B. S., and Luizzi, A. Analysis of multi-channel gamma ray spectrometer data with adjustment for gain and baseline discrepancies. Nuclear Instr. Meth. 57: 229 (1967).
4. Pasternack, B. S., and Luizzi, A. Some experimental sampling results for regression analysis applied to gamma ray spectrometer data, II. Biometrics 24: 353 (1968).
5. Rummel, R. J. Applied Factor Analysis. Northwestern University Press, Evanston, Ill., 1977.

Comments on "Experimental Approaches for Exposure to Sized Glass Fibers"

William E. Smith (*Fairleigh-Dickinson Univ., Madison, N.J. 07940*): We have carried out experiments with glass fibers injected into the pleural space of hamsters. No tumors were induced by ordinary insulation-grade fibers about 5 μm in diameter. We have gotten tumors with fibers 1 to 1.5μm in diameter, but only when we used relatively long fibers. With these very thin fibers, we got tumors with preparations containing more than 30% of fibers longer than 20 μm. We got no tumors with preparations in which only about 2% of fibers were longer than 10μm.